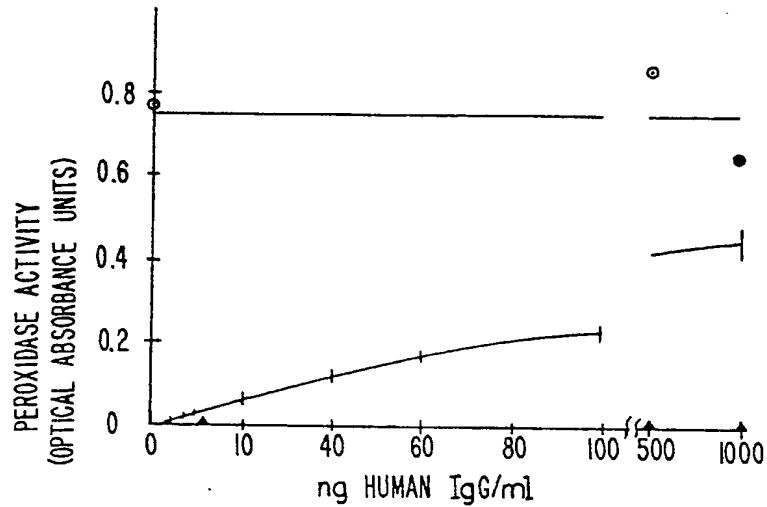




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(54) Title: SOLID PHASE IMMUNOASSAY USING IMMUNOREAGENTS IMMOBILIZED ON INERT SYNTHETIC RESIN SURFACES



(57) Abstract

A solid phase immunoassay comprises the steps of a) immobilizing an immunoreagent on the surface of a carrier comprised of an inert synthetic resin selected from the group consisting of polyimides and polyfluorinated synthetic resins, b) contacting the immunoreagent with a complementary immunoreagent whereby an immunocomplex immobilized on said carrier is formed, c) quantitating the immobilized immunocomplex. An element useful in conducting this solid phase immunoassay is prepared by a process of treating the surface of an article comprised of a synthetic polymer selected from the group consisting of polyimides and polyfluorinated synthetic resins to make it adsorptive of an immunoreagent which comprises the steps of a) thoroughly rinsing the surface with a water-miscible organic solvent, b) thoroughly rinsing the surface with water.

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5 TITLE: SOLID PHASE IMMUNOASSAY USING IMMUNO-
 REAGENTS IMMOBILIZED ON INERT SYNTHETIC
 RESIN SURFACES

BACKGROUND OF THE INVENTION

10

Field of the Invention:

This invention relates to solid phase immunoassays using immobilized immunoreagents and more particularly to elements for solid phase immunoassay comprising 15 immunoreagents immobilized on carriers made of inert synthetic resins. The invention also relates to methods of preparing synthetic resin surfaces for use as carriers for immunoreagents.

20

Description of the Prior Art:

Immunoassay using immobilized immunoreagents is an analytical method widely used in biochemical analysis. In the conventional procedure an immunoreagent, e. g., an antigen or antibody, is first immobilized on the 25 surface of an analytical element, e. g., a test tube, a rod or stick, beads of glass or plastic, or the like. The immobilized immunoreagent is then contacted with an analyte solution containing a complementary immunoreagent, whereby an immobilized immunocomplex is formed. The immobilized immunocomplex can then be easily separated from the unreacted analyte solution, e. g., by 30 simply removing the analyte solution by aspiration, decantation, or the like, preferably with repeated washing of the immobilized immunocomplex. The separated immobilized immunocomplex may then be subjected to further processing to quantitate the amount of immunocomplex. For example, in a radioimmunoassay, the 35

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5 amount of adsorbed complex may be determined by counting radioactive disintegrations; in an enzyme-linked
immunosorbent assay (ELISA) the adsorbed complex which
has an enzyme coupled thereto, is contacted with a
substrate for the enzyme to produce a detectable prod-
10 uct; in an immunofluorescent assay, the fluorescent
intensity of a fluorescent substance linked to the
immunocomplex may be measured, or the like.

15 Among the many different materials which have been
used as carriers for an immunoreagent are glass, metal,
and various plastics such as polystyrene, polyvinyl
chloride, silicone resins, polyethylene and the like.
In some cases the immunoreagent has been immobilized by
covalent bonding to the carrier, while in other cases
adsorption of the immunoreagent has proved adequate.
20 Synthetic resin carriers have been widely used because
of their economy and convenience and ease of handling,
but problems remain in immobilizing enough immunore-
agent on their surfaces for maximum sensitivity of the
immunoassay, especially when the reagent is merely
25 adsorbed onto the surface. Furthermore, the synthetic
resin carriers used previously are inadequate for some
of the newer immunoassay techniques such as thermochem-
iluminescent immunoassay, which requires that the car-
rier with immobilized immunocomplex be heated to rela-
30 tively high temperatures, i. e., 200°C to 300°C. The
conventional plastic carriers cannot be used under
these conditions because they soften or even melt at
such temperatures.

35 On the other hand, certain plastics are known
which can be used at temperatures of 200° to 300°C
without melting or deformation, for example, polyimide
synthetic resins, and polytetrafluoroethylene (PTFE)
and related fluorinated olefin polymers. However,
these resins are very inert and non-adhesive, and it

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5 has been thought that satisfactory adsorption of immunoagents to such surfaces was not possible.

Prior workers in this field do not appear to have attempted to use a polyimide as a carrier for an immunoagent in a solid phase immunoassay.

10 Some attempts have been made to use PTFE as a carrier for solid phase immunoassay, but the procedures have been unsuccessful or have had serious drawbacks.

15 Shekarchi, et al., J. Clin. Microbiology 16(6), 1012-1018 (Dec., 1982) disclose an immunoassay procedure wherein an immunoreagent is immobilized on a small stick, i. e., "microstick", for easy manipulation of the reagent and the immunocomplex. While a number of materials were investigated for use in such microsticks, including stainless steel, nylon, polycarbonate, 20 polystyrene and PTFE, it was found that the PTFE, cleaned by the conventional procedure of rinsing with 6 N HCl, adsorbed very little of the immunoreagent as compared with the other materials and could not be used as a base for the immunoreagent until it had been 25 coated with polycarbonate or nitrocellulose.

30 German Offenlegungsschrift 32 00 822, published July 21, 1983, discloses a method for activating the surface of PTFE articles, in order to bond immunoreagents covalently, by contacting the PTFE surface with an ammoniacal solution of sodium, followed by treatment with carbodiimide. The process was apparently attempted because it was found that adsorption of the immunoreagent on PTFE was unsatisfactory. This process is complex and uses reagents which are difficult to 35 handle and even dangerous. Furthermore, there is some question whether the procedure of this German application actually can immobilize a useful amount of immuno-reagent on PTFE.

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5 Hence, a need has continued to exist for improved
immunoassay procedures using immunoreagents immobilized
on inert carriers such as polyimides and fluorinated
polymers and for a practical method of immobilizing
immunoreagents on the surfaces of such synthetic res-
10 ins.

SUMMARY OF THE INVENTION

It has now been found that a solid phase immunoassay element comprising an immunoreagent adsorbed onto
15 the surface of a heat resistant synthetic resin such as
a polyimide or PTFE can be prepared by adsorbing the
immunoreagent onto the surface by simply incubating the
surface in an aqueous solution of the immunoreagent,
provided that the surface of the synthetic resin has
20 first been cleaned by thorough rinsing with a water-
miscible organic solvent followed by thorough rinsing
with water.

The invention further comprises a solid phase
immunoassay procedure using an immunoreagent immobilized
25 on a carrier made of a polyimide or a polyfluorinated synthetic resin.

The invention further comprises a process for
treating the surface of an article comprised of a synthetic polymer selected from the group consisting of
30 polyimides and polyfluorinated synthetic resin polymers
to make it receptive to adsorption of an immunoreagent
this process comprising the steps of

- a) thoroughly rinsing the surface with a water-miscible organic solvent, and
- 35 b) thoroughly rinsing the surface with water.

An immunoreagent may then be adsorbed onto the
surface of the cleaned synthetic resin by contacting
the surface with an aqueous solution of the immunoreagent.

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5 Thus, it is an object of the invention to provide a method of solid phase immunoassay using an immunoreagent immobilized on the surface of an inert synthetic resin.

10 A further object is to provide an immobilized immunoreagent.

15 A further object is to provide an immunoreagent immobilized on a synthetic resin surface, where the synthetic resin is a polyimide or a polyfluorinated synthetic resin.

20 A further object is to provide a method for treating an inert synthetic resin surface to make it adsorptive to immunoreagents.

25 A further object is to provide a method of preparing an immobilized immunological reagent.

30 A further object is to provide a method of immobilizing an immunological reagent on a polyimide synthetic resin surface.

35 A further object is to provide a method of immobilizing an immunological reagent on a polyfluorinated polymer surface.

40 Further objects of the invention will become apparent from the description of the invention which follows.

45 BRIEF DESCRIPTION OF THE DRAWINGS

50 Figure 1 illustrates the results of the solid phase enzyme-linked immunosorbent assay of Example 3 using a conventional polystyrene carrier.

55 Figure 2 illustrates the results of the solid phase enzyme-linked immunosorbent assay of Example 3 using a polyimide resin carrier.

60 Figure 3 illustrates the results of the solid phase enzyme-linked immunosorbent assay of Example 3 using a PTFE carrier.

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5

DETAILED DESCRIPTION OF THE INVENTION
AND PREFERRED EMBODIMENTS

10 The solid phase immunoassay of the invention using an immunoreagent immobilized on an inert resin surface of a polyimide or a polyfluorinated synthetic resin is capable of giving greater sensitivity than assays using immunoreagents immobilized on conventional carriers such as polystyrene and the like.

15 Solid phase immunoassay elements comprising an immunoreagent immobilized on a carrier surface of a polyimide or polyfluorinated synthetic resin are essential to the practice of the immunoassay of the invention. Such elements are useful in the practice of conventional immunoassays, but are especially useful for thermochemiluminescence immunoassays which require 20 that the carrier with an immobilized immunocomplex be heated to relatively high temperatures, e. g., 200°C to 300°C.

25 The solid phase immunoassay elements of this invention use carriers comprised of a polyimide or a polyfluorinated synthetic resin. The polyimide resins are inert heat-resistant synthetic resins which are characterized by the presence of the phthalimide structure in the backbone. A preferred polyimide is a condensation polymer of pyromellitic acid and bis(4-aminophenyl)oxide, sold by E. I. du Pont de Nemours & Co. under the name Kapton. The process may also employ polyfluorinated synthetic resins having surfaces of low surface energy which have hitherto been thought to be too non-adhesive for adsorption of immunoreagents. Such polymers include polymers of perfluorinated carbonates, polymers of perfluorinated epoxy compounds and especially addition polymers of ethylenically unsaturated hydrocarbons such as polytetrafluoroethylene, poly(chlorotrifluoroethylene) and

5 the like. These materials are manufactured by a number
of suppliers, e. g., by the Du Pont Co., under the name
Teflon .

10 The water-miscible organic solvent used in the
preparation of the immunoassay elements of the inven-
tion may be any solvent which can produce the requisite
cleanliness of the surface of the synthetic resin, and
is sufficiently miscible with water to be completely
removed by subsequent thorough rinsing with water.
Such solvents include lower aliphatic alcohols, e. g.,
15 C₁-C₄ aliphatic alcohols such as methanol, ethanol, n-
propanol, isopropyl alcohol, n-butanol, isobutanol,
sec.-butyl alcohol and tert.-butyl alcohol; lower ali-
phatic ketones, e. g., acetone, methyl ethyl ketone,
and the like, dioxan, dimethylformamide, dimethyl sulf-
20 oxide, acetonitrile, lower (C₁-C₄) glycols such as
ethylene glycol and propylene glycol, and lower aliphatic
ethers having a total of about 3 to 6 carbon atoms,
e. g., 2-methoxyethanol, 2-ethoxyethanol and the like.
Mixtures of solvents may also be used. Preferred sol-
vents are lower aliphatic alcohols, and a most pre-
25 ferred solvent is ethanol.

30 The solvent rinsing step is preferably conducted
by contacting the surface of the synthetic resin with
clean solvent for a period of time required to thor-
oughly clean the surface. The exact experimental con-
ditions may vary with the solvent chosen, but the eval-
uation of the results in order to choose the proper
condition may be simply carried out by using the sur-
face in a standard immunoassay. A surface which has
35 been inadequately cleaned will poorly adsorb the immu-
noreagent and manifest this poor adsorption by a low
sensitivity in the immunoassay. It is preferable to
have the solvent which contacts the synthetic resin
surface as free as possible of contaminants. This can

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5 be accomplished by continuously supplying fresh solvent
to the surface, e. g., in a flowing system, or by fre-
quent changes of solvent when the cleaning is conducted
by a batch process. The solvent will ordinarily be
10 used in an anhydrous state for maximum solvent power
for organic contaminants on the resin surface. How-
ever, the invention is also intended to include the use
of solvents containing small amounts of water which do
not seriously interfere with the solvent properties of
the solvents toward organic surface contaminants.

15 It is preferred to conduct the solvent rinsing
step by contacting the surface with the solvent at its
boiling temperature. Accordingly water-miscible sol-
vents having boiling points between about 40°C and
about 200°C are preferred. The solvent rinsing step is
20 therefore conveniently carried out by placing the arti-
cles to be coated, e. g., disks of the polymer to be
used in immunosorbent assays, in a flask containing the
solvent and heating to reflux temperature. The time of
the solvent rinsing step will also vary depending on
25 the solvent and its temperature. A relatively short
contact time, e. g., one-half hour, may produce a sur-
face which will adsorb some immunoreagent. However, it
is preferable to keep the surface in contact with the
solvent for a relatively long period of time, e. g.,
30 one or two days, in order to assure maximum cleaning
and maximum adsorption of immunoreagent, which assures
the highest sensitivity of the immunoassay. It is also
preferable to change the solvent several times in the
course of the rinsing step to insure maximum cleanli-
35 ness of the solvent in the final portion of the rinsing
step.

A preferred procedure is to contact the surface of
the synthetic resin carrier with ethanol at refluxing

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5 temperature for a period of one to two days with frequent changes of solvent.

10 After the completion of the solvent cleaning step, the solvent-rinsed surface is then thoroughly rinsed with pure water, e. g., distilled or deionized water, to remove all traces of the solvent. Again, the preferable procedure is to immerse the articles in boiling water in a refluxing apparatus, for several hours, with several changes of water. The presence of residual solvent on the surface of the synthetic resin interferes with the adsorption of the immunoreagent and hence insufficient water rinsing is readily detected by the practitioner by simple use of a standard immunoassay. Evidently, the necessary length of the water rinsing step and the number of changes of fresh water can be easily determined by the practitioner using this criterion.

25 If desired the carriers may be dried after the water rinsing step. The drying is preferably conducted by draining the water from the carriers and allowing the residual water to evaporate. It is preferable to assure complete dryness, especially when the carriers are to be used for a thermochemiluminescent assay, by drying in an oven at an elevated temperature, e. g., 200°C to 300°C, for an extended period of time. Preferred drying conditions are 300°C overnight.

30 The articles prepared by the cleaning process, either immediately after the rinsing step, without drying the surface, or after the surface has been dried, may then be coated with an immunoreagent by a relatively conventional adsorption step wherein the articles are contacted with an aqueous solution of the immunoreagent in a suitable buffer for a period of time to allow physical adsorption to occur to a sufficient extent. The basic process for this adsorption step is

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5 disclosed by Catt, U. S. Patent 3,646,346. For example, the article may be contacted with an aqueous solution of an antibody at a concentration of 0.1 mg/ml in 0.1 M tris(hydroxymethyl)aminomethane (Tris) at a pH of about 7.6 at 4°C for an extended period of time, e. g. 10 2 or more days. The coated articles are then thoroughly washed with distilled water and are ready for use in immunoassay procedures.

15 Any immunoreagent conventionally used in immunoassay can be adsorbed to the surface of the inert synthetic resin carrier used in this invention. Ordinarily, the immunoreagent will be an antibody or an antigen and generally the immunoreagent will be a proteinaceous material.

20 While the preferred conditions are given above, effective ranges are rather broad, and it will be understood by those skilled in the art that the conditions may be varied, provided that the essential steps are performed. The success of the treatment under a given set of conditions is easily evaluated by 25 simply measuring the amount of immunoreagent absorbed, e. g., by use in a conventional standard immunoassay.

The invention will be illustrated by the following examples which are not intended to be limiting. In the examples all parts and percentages are by weight.

30

Example 1

This example illustrates the surface treatment process of process of this invention and immobilization of immunoreagent.

35 About 150 disks of a polyimide synthetic resin (Kapton 500H, manufactured by the du Pont company) having a diameter of 9 mm and a thickness of 125 micrometers were placed in a flask equipped with a reflux condenser, covered with ethanol, and rinsed with ethanol at reflux temperature for a period of one day with

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5 several changes of the ethanol solvent. The disks were then rinsed with distilled water at reflux temperature for several hours with several changes of distilled water. The disks were then removed from the flask and dried in an oven at a temperature of 300°C overnight.

10 The dried disks were then placed in 40 ml of a solution of 0.1 mg/ml of antibody in 0.1 M Tris buffer (pH 7.6) and 0.02 % NaN_3 , at a temperature of 4°C. The disks were gently shaken during the coating procedure which was continued for a period of 2 days. The coated

15 disks were then washed in distilled water and could be preincubated, if desired, to prepare them for use in immunoassay by incubating them in an aqueous solution of 4 % bovine serum albumin (BSA) in a 10 mM Tris buffer (pH 8.0), 0.15 M NaCl, 0.05 % polyoxyethylene sorbitan surfactant (Tween 20) (Buffer A) for at least

20 one hour.

Example 2

25 The procedure of Example 1 was used to prepare and coat about 150 disks of polytetrafluoroethylene having a diameter of 9 mm and a thickness of 500 micrometers.

Example 3

30 This example illustrates a comparison of ELISA conducted with the immunological reagent adsorbed by conventional procedures on a polystyrene microtiter plate and on carriers coated according to this invention.

35 Disks of polyimide or of polytetrafluoroethylene were coated by the procedure of Example 1 with goat, antihuman Immunoglobulin G (IgG) (7S) (Nordic), human IgG (Sigma) and BSA (Sigma). Polystyrene microtiter plates (Costar, Holland) were coated for one week with the same antibodies and with BSA by contacting them for a period of one week with a solution of 0.1 mg protein per ml, 0.1 M carbonate buffer, pH 9.6, at a tempera-

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5 ture of 4°C. Each well of the plates contained 0.1 ml of the solution. After the different carrier polymers were coated with the different immunoreagents, the solid phase immunoassay procedures were identical.

10 The coated carriers were preincubated for one hour with 0.2 ml of Buffer A. After washing with distilled water, the disks were incubated with 0.2 ml human IgG standards (0; 1; 2.5; 5; 7.5; 10; 20; 40; 60; 100; 500; 1000 ng human IgG per ml of Buffer A) for one hour at 37°C while shaking in a waterbath. Subsequently, the 15 disks were again washed with distilled water by decantation and incubated with 0.2 ml of an aqueous solution containing 1 microgram of peroxidase labeled goat-antihuman conjugate (Nordic) per milliliter of Buffer A at 37°C in a waterbath with shaking. After removal of 20 unbound conjugate, peroxidase activity on the disks was measured by means of the conversion of ortho-phenylenediamine to a colored product. Carriers coated with immunoreagent were incubated in the dark with 0.2 ml of M-phosphate buffer, pH 5.0, 0.0045 % H_2O_2 , $2 \cdot 10^{-3}$ M 25 ortho-phenylenediamine HCl (UCB) for one hour. Color development was stopped by adding of 0.5 ml of 1 N H_2SO_4 . The optical density of the color was measured at 495 nm with a Zeiss spectrophotometer.

30 Figure 1 shows the results of an immunoassay on a polystyrene carrier. Figure 2 shows the results of the same immunoassay using the polyimide (Kapton 500H) disks coated by the process of this invention. Figure 35 3 shows the results of the same immunoassay performed using PTFE disks coated by the process of this invention.

In the figures the symbols I indicate the values measured for the series of human IgG standards. Each data bar represents a triplicate measurement. The vertical length of the bar represents the range of the

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5 repeated determinations. Note that at a concentration below 20 ng/ml the measurements are so reproducible that the data bar has hardly any vertical length. The triangles indicate the measured values for the negative control (BSA) and the circles represent the measured 10 values for the positive control (disks coated with pure antigen, human IgG).

Figures 2 and 3 show that the peroxidase activity of the adsorbed immunocomplex is substantially greater for the polyimide and PTFE carriers prepared according 15 to this invention than for the conventional polystyrene carrier. Accordingly, is evident from the results of the experiment that the inert synthetic resin surfaces prepared and coated with immunoreagent by the process of this invention yield results superior to the conventional immunoassay using polystyrene microtiter plates.

Example 4

This example illustrates the results of comparative immunoassays using polyimide carriers prepared by cleaning processes different from that of this invention.

A. Acidic cleaning treatment.

Kapton disks were contacted with 98 % sulfuric acid for one minute at room temperature, washed with distilled water, and immediately (without drying) incubated with either human serum albumin (antigen) or human IgG (non-antigen). The incubated disks were then contacted with an aqueous solution of peroxidase-labeled rabbit-anti-human serum albumin (antibody). It was found that the amount of antibody bound to the carrier was the same for both the antigen-coated carrier and the carrier coated with non-antigen protein. Accordingly the antibody binding of the polyimide carrier prepared by an acid cleaning treatment is completely non-specific, and such a carrier is useless for

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5 solid phase immunoassay.

B. Basic cleaning treatment

Polyimide disks were treated with 5 % KOH in distilled water for 5 minutes at room temperature, then washed a few times with distilled water. Analytical 10 disks were then incubated with a series of human IgG standards of various concentrations according to the procedure of Example 3, while control disks were coated by incubation with a solution of bovine serum albumin (BSA). The immunoassay was conducted by the procedure 15 of Example 3. It was found that the blank value of bound peroxidase activity (i. e., for the disks coated with BSA) was very high, about 0.80 to about 1.0 optical absorbance units. While the measured values of bound peroxidase activity for the disks coated with 20 human IgG were measurably greater than the blank value, it is evident that such a high blank value produces an assay having inferior sensitivity and accuracy.

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Example 5

5 This example illustrates the specific nature of
the pretreatment process of this invention
10 Glass disks about 1.0 cm in diameter were treated
by the process of Example 1 and used in an immunoassay
as described in Example 3. It was found that the blank
15 value (BSA coated disks) was rather high, about 0.25
optical absorbance units, while the measured value for
an analyte concentration of 100 ng/ml (human IgG) was
only about 0.40, i. e., only about 0.15 optical absorb-
ance units greater than the blank value. The same
15 process applied to glass beads about 5-7 mm in diameter
gave even poorer results; the beads appeared to have no
specific binding power after being subjected to the
conventional adsorption process to coat them with an
immunoreagent. Such results indicate that this process
20 is not suitable for use with glass carriers. Accord-
ingly, it is evident that the process of the invention
is not a general cleaning process, but rather a clean-
ing process specially adapted to cleaning and preparing
carriers of the claimed synthetic resins.
25 The invention having now been fully described, it
should be understood that it may be embodied in other
specific forms or variations without departing from its
spirit or essential characteristics. Accordingly, the
embodiments described above are to be considered in all
30 respects as illustrative and not restrictive, the scope
of the invention being indicated by the appended claims
rather than by the foregoing description, and all
changes which come within the meaning and range of
equivalency of the claims are intended to be embraced
35 therein.

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5 WHAT IS CLAIMED IS:

1. A solid phase immunoassay comprising the steps of
 - a) immobilizing an immunoreagent on the surface of a carrier comprised of an inert synthetic resin selected from the group consisting of polyimides and polyfluorinated synthetic resins,
 - b) contacting said immunoreagent with a complementary immunoreagent whereby an immunocomplex immobilized on said carrier is formed,
 - c) quantitating said immobilized immunocomplex.
2. The immunoassay of Claim 1 wherein said synthetic resin is a polyimide.
3. The immunoassay of Claim 2 wherein said polyimide is a polymer of pyromellitic acid and bis(4-aminophenyl)oxide.
4. The immunoassay of Claim 1 wherein said synthetic resin is a polyfluorinated synthetic resin.
5. The immunoassay of Claim 4 wherein said polyfluorinated synthetic resin is a polymer of a polyfluorinated ethylenically unsaturated hydrocarbon.
6. The immunoassay of Claim 5 wherein said polyfluorinated synthetic resin is polytetrafluoroethylene.
7. The immunoassay of Claim 1 wherein said immunoreagent is a protein.
8. The immunoassay of Claim 1 wherein said immunoreagent is an antibody.

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5 9. The immunoassay of Claim 1 wherein said immunoassay is an antigen.

10 10. An element for use in a solid phase immunoassay comprising an immunoreagent immobilized on the surface of a carrier comprised of an inert synthetic resin selected from the group consisting of polyimides and polyfluorinated synthetic resins.

15 11. The element of Claim 10 wherein said synthetic resin is a polyimide.

20 12. The element of Claim 11 wherein said polyimide is a polymer of pyromellitic acid and bis(4-aminophenyl)oxide.

13. The element of Claim 10 wherein said synthetic resin is a polyfluorinated synthetic resin.

25 14. The element of Claim 13 wherein said polyfluorinated synthetic resin is a polymer of a polyfluorinated ethylenically unsaturated hydrocarbon.

15. The element of Claim 14 wherein said polyfluorinated synthetic resin is polytetrafluoroethylene.

30 16. The element of Claim 10 wherein said immunoreagent is a protein.

17. The element of Claim 10 wherein said immunoreagent is an antibody.

35 18. The element of Claim 10 wherein said immunoreagent is an antigen.

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5 19. A process for treating the surface of an article comprised of a synthetic polymer selected from the group consisting of polyimides and polyfluorinated synthetic resins to make it receptive to adsorption of an immunoreagent, said process comprising the steps of

10 a). thoroughly rinsing the surface with a water-miscible organic solvent,

 b) thoroughly rinsing the surface with water.

15 20. The process of Claim 19 wherein said synthetic resin is a polyimide.

20 21. The process of Claim 20 wherein said polyimide is a polymer of pyromellitic acid and bis(4-aminophenyl)oxide.

25 22. The process of Claim 19 wherein said synthetic resin is a polyfluorinated synthetic resin.

25 23. The process of Claim 22 wherein said polyfluorinated synthetic resin is a polymer of a polyfluorinated ethylenically unsaturated hydrocarbon.

30 24. The process of Claim 23 wherein said polyfluorinated synthetic resin is polytetrafluoroethylene.

30 25. The process of Claim 19 wherein said solvent is a C₁-C₄ aliphatic alcohol.

35 26. The process of Claim 25 wherein said alcohol is ethanol.

35 27. The process of Claim 19 wherein said solvent rinsing is carried out at a temperature equal to boiling point of the solvent.

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5 28. The process of Claim 19 wherein said solvent rinsing step is carried out for a period of one-half hour to four days.

10 29. The process of Claim 28 wherein said solvent rinsing step is carried out for at least one day.

15 30. The process of Claim 19 wherein said water rinsing is carried out at a temperature equal to the boiling point of water.

15 31. The process of Claim 19 additionally comprising the step of
b') drying said surface after step b).

20 32. The process of Claim 31 wherein said drying step is carried out at a temperature greater than 200°C.

25 33. The process of Claim 32 wherein said drying step is carried out at a temperature of about 300°C.

30 34. A process for preparing an element suitable for use in a solid phase immunoassay, said process comprising the steps of

30 a) thoroughly rinsing a surface of an article comprised of a synthetic resin selected from the group consisting of polyimides and polyfluorinated synthetic resins with a water-miscible organic solvent,
b) thoroughly rinsing the surface with water, and
c) contacting the surface with an aqueous solution of an immunoreagent.

35 35. The process of Claim 34 wherein said synthetic resin is a polyimide.

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5 36. The process of Claim 35 wherein said polyimide is a polymer of pyromellitic acid and bis(4-aminophenyl)oxide.

10 37. The process of Claim 34 wherein said synthetic resin is a polyfluorinated synthetic resin.

15 38. The process of Claim 37 wherein said polyfluorinated synthetic resin is a polymer of a polyfluorinated ethylenically unsaturated hydrocarbon.

15 39. The process of Claim 38 wherein said polyfluorinated synthetic resin is polytetrafluoroethylene.

20 40. The process of Claim 34 wherein said solvent is a C₁-C₄ aliphatic alcohol.

25 41. The process of Claim 40 wherein said alcohol is ethanol.

25 42. The process of Claim 34 wherein said solvent rinsing is carried out at a temperature equal to boiling point of the solvent.

30 43. The process of Claim 34 wherein said solvent rinsing step is carried out for a period of one-half hour to four days.

35 44. The process of Claim 34 wherein said solvent rinsing step is carried out for at least one day.

35 45. The process of Claim 34 wherein said water rinsing is carried out at a temperature equal to the boiling point of water.

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5 46. The process of Claim 34 additionally comprising the step of

 b') drying said surface after step b) and before step c).

10 47. The process of Claim 46 wherein said drying step is carried out at a temperature greater than 200°C.

15 48. The process of Claim 47 wherein said drying step is carried out at a temperature of about 300°C.

20 49. A carrier for an immunoreagent to be used in a solid phase immunoassay, said carrier comprising an article having a surface comprised of a synthetic resin selected from the group consisting of polyimides and polyfluorinated synthetic resins, said surface having been treated by a process comprising the steps of

 a) thoroughly rinsing the surface with a water-miscible organic solvent, and

25 b) thoroughly rinsing the surface with water.

50. The carrier of Claim 49 wherein said synthetic resin is a polyimide.

30 51. The carrier of Claim 50 wherein said polyimide is a polymer of pyromellitic acid and bis(4-aminophenyl)oxide.

35 52. The carrier of Claim 49 wherein said synthetic resin is a polyfluorinated synthetic resin.

53. The carrier of Claim 52 wherein said polyfluorinated synthetic resin is a polymer of a polyfluorinated ethylenically unsaturated hydrocarbon.

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5 54. The carrier of Claim 53 wherein said poly-
fluorinated synthetic resin is polytetrafluoroethylene.

10 55. The carrier of Claim 49 wherein said solvent
is a C₁-C₄ aliphatic alcohol.

15 56. The carrier of Claim 55 wherein said alcohol
is ethanol.

20 57. The carrier of Claim 49 wherein said solvent
rinsing is carried out at a temperature equal to boil-
ing point of the solvent.

25 58. The carrier of Claim 49 wherein said solvent
rinsing step is carried out for a period of one-half
hour to four days.

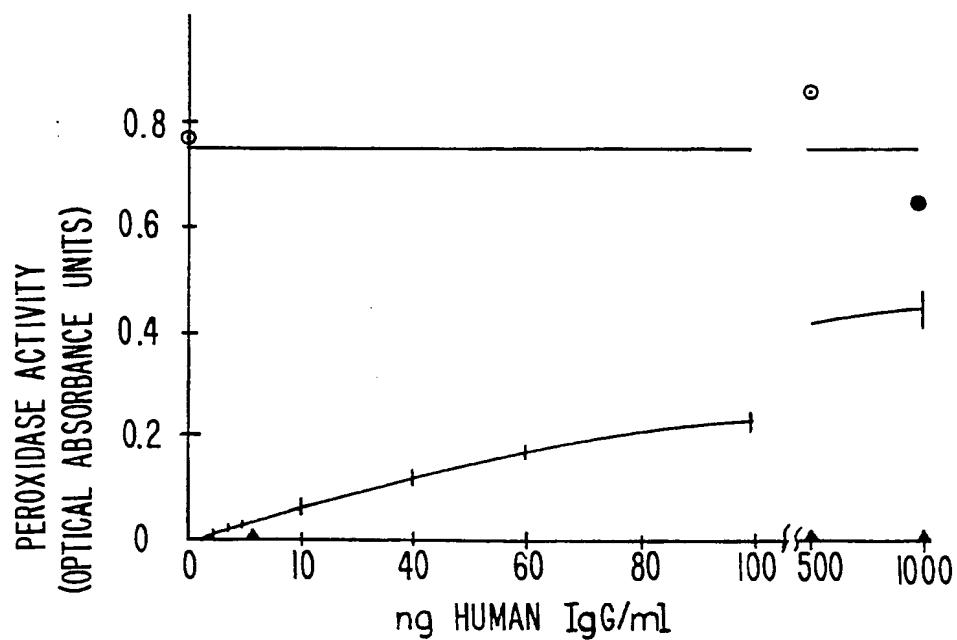
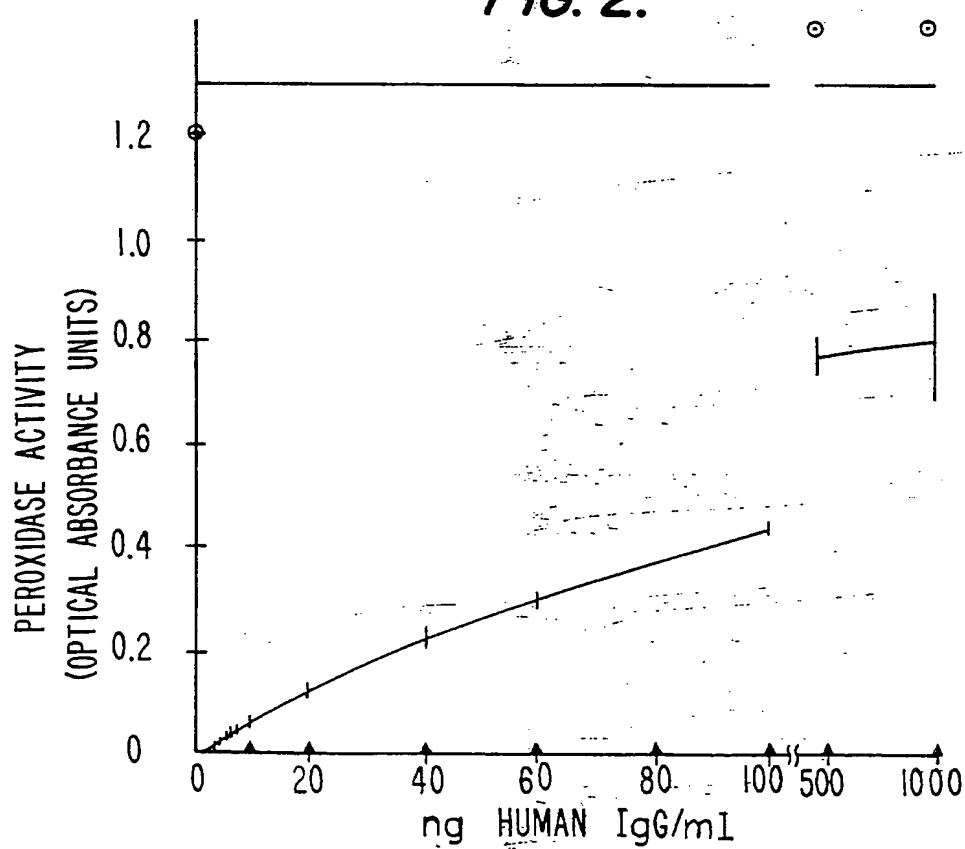
30 59. The carrier of Claim 49 wherein said solvent
rinsing step is carried out for at least one day.

35 60. The carrier of Claim 49 wherein said water
rinsing is carried out at a temperature equal to the
boiling point of water.

40 61. The carrier of Claim 49 additionally compris-
ing the step of
b') drying said surface after step b).

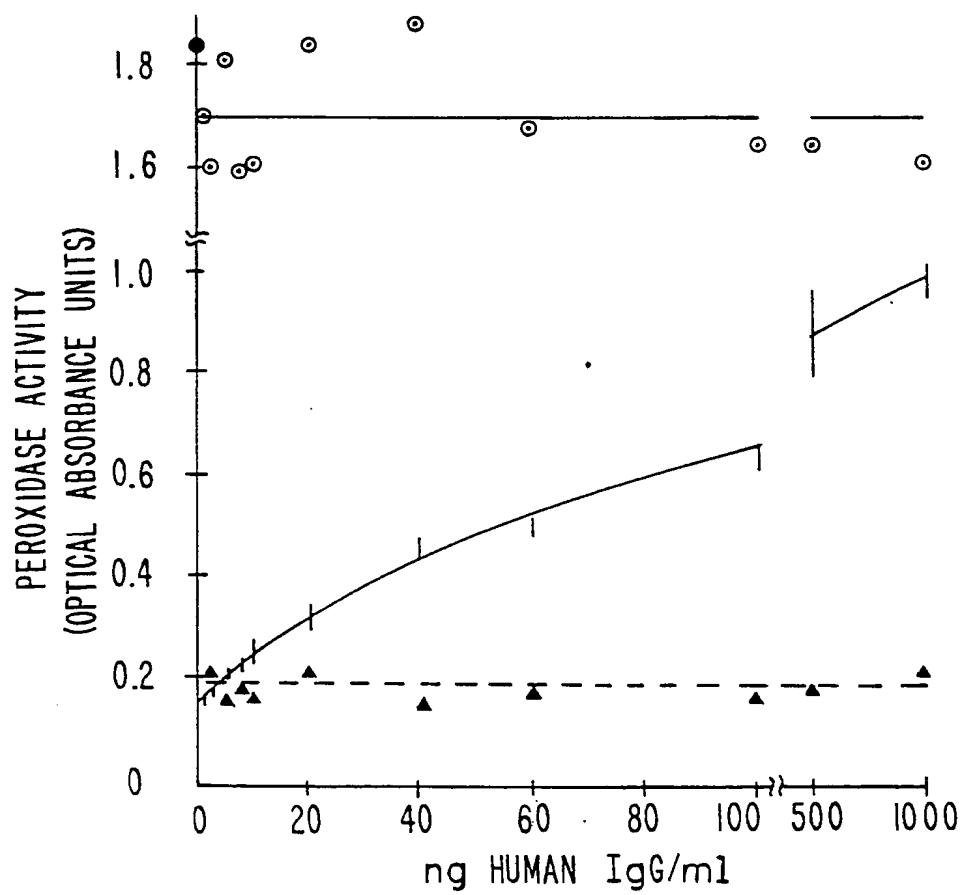
45 62. The carrier of Claim 61 wherein said drying
step is carried out at a temperature greater than
200°C.

50 63. The carrier of Claim 62 wherein said drying
step is carried out at a temperature of about 300°C.

1/2 **FIG. 1.****FIG. 2.**

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FIG. 3.



INTERNATIONAL SEARCH REPORT

International Application No PCT/US85/02504

I. CLASSIFICATION OF SUBJECT MATTER (If several classification symbols apply, indicate all) ¹⁵

According to International Patent Classification (IPC) or to both National Classification and IPC

US Cl. 436-531, 534, 823, 824; 435-7
Int Cl. IV- G 01 N 33/545, 546, 53

II. FIELDS SEARCHED

Minimum Documentation Searched ¹⁶

| Classification System | Classification Symbols |
|-----------------------|------------------------|
| US Cl. | 436-531, 534, 823, 824 |
| | 435-7 |

Documentation Searched other than Minimum Documentation
to the Extent that such Documents are Included in the Fields Searched ⁶

Dialog 2 files 399, 5, 55, 154, 76, 73, 72, 350, 351
Lexpat (Mead Data)

III. DOCUMENTS CONSIDERED TO BE RELEVANT ¹⁴

| Category ¹⁶ | Citation of Document, ¹⁶ with indication, where appropriate, of the relevant passages ¹⁷ | Relevant to Claim No. ¹⁶ |
|------------------------|--|-------------------------------------|
| X, Y | US, N, "Coupling of proteins and haptens on Teflon for immunological assay", Chemical abstracts, Vol. 99 issued September 1983, abstract number 101950j, Von Klitzing et al (entire abstract). | 1, 4-10, 13-18, 49, 52-54, 61 |
| X | US, A, 4,017,597, published 12 April 1977, Reynolds, see column 10, lines 14-20. | 1, 4-10, 13-18, 49, 52-54, 61 |
| X | US, A, 4,444,879, published 24 April 1984, Foster et al, see column 3, lines 32-37 | 1, 4-10; 13-18, 49, 52-54, 61 |
| X | US, A, 4,360,358, published 23 November 1982 Sharma, see column 5, line 58- column 6, line 17 | 1, 4-10, 13-18, 49, 52-54, 61 |
| X | US, A, 4,357,142, published 2 November 1982, Schall, Jr. et al, see column 6, lines 37-64 | 19, 34 |

* Special categories of cited documents: ¹⁵

"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search ¹⁸

07 March 1986

Date of Mailing of this International Search Report ¹⁹

12 MAR 1986

International Searching Authority ¹

ISA/US

Signature of Authorized Officer ²⁰

Christine M. Nucker